

What is claimed is:

- 1 1. A test strip adapted to receive a sample and detect an analyte therein, the test
2 strip comprising:
 - 3 a buffer addition zone to which a buffer may be added;
 - 4 an absorbent zone proximal to the buffer addition zone;
 - 5 one or more test zones distal to the buffer addition zone, at least one of the test
6 zones including a first analyte binding agent immobilized therein which is capable of
7 binding to the analyte to be detected;
 - 8 a terminal buffer flow zone distal to the one or more test zones, the absorbent
9 zone being positioned relative to the buffer addition zone and having an absorption
10 capacity relative to the other zones of the test strip such that when a volume of buffer
11 within a predetermined buffer volume range for the test strip is added to the buffer
12 addition zone, a distal diffusion front of the buffer diffuses from the buffer addition
13 zone to a distal diffusion point within the terminal buffer flow zone and then diffuses
14 proximal relative to the one or more test zones; and
 - 15 a sample addition zone distal to the terminal buffer flow zone to which a
16 sample may be added.
- 1 2. A test strip according to claim 1 wherein the test strip further includes a zone
2 distal to the terminal buffer flow zone which includes a second analyte binding agent
3 which is capable of binding to the analyte and diffusing to the one or more test zones.
- 1 3. A test strip according to claim 1 wherein the second analyte binding agent is
2 capable of binding to components in the sample other than the analyte.
- 1 4. A test strip according to claim 1 wherein the second analyte binding agent
2 does not bind to components in the sample other than the analyte.

1 5. A test strip according to claim 1 wherein the second analyte binding agent is
2 labeled with a detectable marker.

1 6. A test strip according to claim 1 wherein the second analyte binding agent is
2 attached to a particle which is capable of diffusing to the one or more test zones.

1 7. A test strip according to claim 1 wherein the zone containing the second
2 analyte binding agent is proximal to the sample addition zone.

1 8. A test strip according to claim 1 wherein the zone containing the second
2 analyte binding agent is the sample addition zone.

1 9. A test strip according to claim 1 wherein the test strip further includes a zone
2 distal to the terminal buffer flow zone which includes a competitive agent which is
3 capable of competing with the analyte to bind to the first analyte binding agent.

1 10. A test strip according to claim 1 wherein the competitive agent binds to the
2 first analyte binding agent and does not bind to components in the sample other than
3 the first analyte binding agent.

1 11. A test strip according to claim 1 wherein the competitive agent is labeled with
2 a detectable marker.

1 12. A test strip according to claim 1 wherein the competitive agent is attached to a
2 particle which is capable of diffusing to the one or more test zones.

1 13. A test strip according to claim 1 wherein the zone containing the competitive
2 agent is proximal to the sample addition zone.

1 14. A test strip according to claim 1 wherein the zone containing the competitive
2 agent is the sample addition zone.

1 15. A test strip according to claim 1 wherein the sample addition zone is
2 positioned relative to the test zones such that sample added to the sample addition
3 zone at the same time as buffer is added to the buffer addition zone reaches the distal
4 diffusion point after the distal diffusion front of the buffer has diffused to the distal
5 diffusion point and begun diffusing in a proximal direction.

1 16. A test strip according to claim 1 wherein the sample addition zone is
2 positioned relative to the test zones such that sample added to the sample addition
3 zone at the same time as buffer is added to the buffer addition zone reaches the test
4 zones after the distal diffusion front diffuses proximal relative to the test zones.

1 17. A test strip according to claim 1 wherein the predetermined buffer volume
2 range is between about 10 and 250 L.

1 18. A test strip according to claim 1 wherein the predetermined buffer volume
2 range is between about 20 and 200 L.

1 19. A test strip according to claim 1 wherein the predetermined buffer volume
2 range is between about 20 and 100 L.

1 20. A test strip according to claim 1 wherein the predetermined buffer volume
2 range is between about 40 and 60 L.

1 21. A test strip according to claim 1 wherein the terminal buffer flow zone has a
2 length from a proximal end to a distal end of between about 3 and 10 mm.

- 1 22. A test strip according to claim 1 wherein the first analyte binding agent does
2 not bind to components in the sample other than the analyte.
- 1 23. A test strip according to claim 1 wherein the first analyte binding agent is
2 selected from the group consisting of antibodies, engineered proteins, peptides,
3 haptens, lysates containing heterogeneous mixtures of antigens having analyte binding
4 sites, ligands and receptors.
- 1 24. A test strip according to claim 1 wherein the test zones further include at least
2 a first control zone with a control binding agent immobilized therein.
- 1 25. A test strip according to claim 1 wherein the test zones further include a first
2 control zone with a control binding agent immobilized therein, and a second control
3 zone with a same control binding agent immobilized therein as the first control zone,
4 the first and second control zones containing a different amount of the control binding
5 agent immobilized therein.
- 1 26. A test strip according to claim 1 wherein the test zones further include a first
2 control zone with a control binding agent immobilized therein, and a second control
3 zone with a same control binding agent immobilized therein as the first control zone,
4 the first and second control zones containing about the same amount of the control
5 binding agent immobilized therein.
- 1 27. A test strip according to claim 1 wherein the test zones further include first and
2 second control zones each with a control binding agent immobilized therein, the first
3 test zone being proximal to the test zone including the first analyte binding agent, the
4 second control zone being distal to the test zone including the first analyte binding
5 agent.

1 28. A method for detecting an analyte in a sample comprising:
2 delivering a buffer to a test strip which causes a distal diffusion front of the
3 buffer to (a) diffuse in a distal direction to one or more test zones, at least one of the
4 test zones including a first analyte binding agent immobilized therein which binds to
5 analyte in the sample, (b) diffuse to a terminal buffer flow zone distal to the one or
6 more test zones, change direction and (c) diffuse to a position proximal to the one or
7 more test zones;
8 delivering a sample to the test strip at a position distal to the terminal buffer
9 flow zone, delivery of the sample causing analyte in the sample to diffuse proximally
10 past the terminal buffer flow zone to the one or more test zones after the distal
11 diffusion front of the buffer diffuses proximal to the one or more test zones, the
12 analyte binding to the first analyte binding agent and becoming immobilized in the
13 test zones; and
14 detecting the analyte immobilized in the test zones.

1 29. A method according to claim 28 wherein
2 the test strip further includes a zone distal to the terminal buffer flow zone
3 which includes a second analyte binding agent which is capable of binding to the
4 analyte,
5 addition of the sample causing the second analyte binding agent to bind to
6 analyte in the sample, binding of the analyte to the first analyte binding agent causing
7 the second analyte binding agent to be immobilized in the test zones, and
8 detecting the analyte immobilized in the test zones comprising detecting the
9 second analyte binding agent.

1 30. A method according to claim 29 wherein the second analyte binding agent is
2 labeled with a detectable marker and detecting the second analyte binding agent
3 comprises detecting the detectable marker.

1 31. A method according to claim 29 wherein the second analyte binding agent is
2 attached to a particle and detecting the second analyte binding agent comprises
3 detecting the particle.

1 32. A method according to claim 29 wherein the zone containing the second
2 analyte binding agent is proximal to the sample addition zone.

1 33. A method according to claim 29 wherein the zone containing the second
2 analyte binding agent is the sample addition zone.

1 34. A method according to claim 28 wherein the sample addition zone is
2 positioned relative to the test zones such that sample added to the sample addition
3 zone at the same time as buffer is added to the buffer addition zone reaches the distal
4 diffusion point after the distal diffusion front of the buffer has diffused to the distal
5 diffusion point and begun diffusing in a proximal direction.

1 35. A method according to claim 28 wherein the sample addition zone is
2 positioned relative to the test zones such that sample added to the sample addition
3 zone at the same time as buffer is added to the buffer addition zone reaches the test
4 zones after the distal diffusion front diffuses proximal relative to the test zones.

1 36. A method according to claim 28 wherein the buffer delivered to the buffer
2 addition zone has a volume between about 10 and 250 L.

1 37. A method according to claim 28 wherein the buffer delivered to the buffer
2 addition zone has a volume between about 20 and 200 L.

1 38. A method according to claim 28 wherein the buffer delivered to the buffer
2 addition zone has a volume between about 20 and 100 L.

- 1 39. A method according to claim 28 wherein the buffer delivered to the buffer
2 addition zone has a volume between about 40 and 60 L.
- 1 40. A method according to claim 28 wherein the buffer delivered to the buffer
2 addition zone comprises the sample delivered to the sample addition zone.
- 1 41. A method according to claim 28 wherein the buffer delivered to the buffer
2 addition zone is the same as the sample delivered to the sample addition zone.
- 1 42. A method according to claim 28 wherein the buffer delivered to the buffer
2 addition zone has substantially the same fluid flow characteristics within the test strip
3 as the sample delivered to the sample addition zone.
- 1 43. A method according to claim 28 wherein the test zones further include a first
2 control zone with a control binding agent immobilized therein, and a second control
3 zone with a same control binding agent immobilized therein as the first control zone,
4 the first and second control zones containing a different amount of the control binding
5 agent immobilized therein.
- 1 44. A method according to claim 28 wherein the test zones further include a first
2 control zone with a control binding agent immobilized therein, and a second control
3 zone with a same control binding agent immobilized therein as the first control zone,
4 the first and second control zones containing about the same amount of the control
5 binding agent immobilized therein.
- 1 45. A method according to claim 28 wherein the test zones further include first
2 and second control zones each with a control binding agent immobilized therein, the
3 first test zone being proximal to the test zone including the first analyte binding agent,
4 the second control zone being distal to the test zone including the first analyte binding
5 agent.

46. A method for detecting an analyte in a sample comprising:

delivering a buffer to a test strip which causes a distal diffusion front of the buffer to (a) diffuse in a distal direction to one or more test zones, at least one of the test zones including a first analyte binding agent immobilized therein which binds to analyte in the sample, (b) diffuse to a terminal buffer flow zone distal to the one or more test zones, change direction and (c) diffuse to a position proximal to the one or more test zones;

delivering a sample to the test strip at a position distal to the terminal buffer flow zone, delivery of the sample causing analyte in the sample to diffuse proximally past the terminal buffer flow zone to the one or more test zones after the distal diffusion front of the buffer diffuses proximal to the one or more test zones, the analyte binding to the first analyte binding agent and becoming immobilized in the test zones; and

delivery of the sample to the test strip also causing a competitive agent to diffuse with the sample to the test zone, the competitive agent being capable of binding to the first analyte binding agent and thus competing with the analyte for binding to the first analyte binding agent.

detecting the competitive agent immobilized in the test zones.

47. A method according to claim 46 wherein the competitive agent is comprised on the test strip in a zone distal to the terminal buffer flow zone.

48. A method according to claim 47 wherein the competitive agent is labeled with a detectable marker.

49. A method according to claim 47 wherein the competitive agent is attached to a particle which is capable of diffusing to the one or more test zones.

1 50. A method according to claim 46 wherein the zone containing the competitive
2 agent is proximal to the sample addition zone.

1 51. A method according to claim 46 wherein the zone containing the competitive
2 agent is the sample addition zone.

1 52. A method according to claim 46 wherein the sample addition zone is
2 positioned relative to the test zones such that sample added to the sample addition
3 zone at the same time as buffer is added to the buffer addition zone reaches the distal
4 diffusion point after the distal diffusion front of the buffer has diffused to the distal
5 diffusion point and begun diffusing in a proximal direction.

1 53. A method according to claim 46 wherein the sample addition zone is
2 positioned relative to the test zones such that sample added to the sample addition
3 zone at the same time as buffer is added to the buffer addition zone reaches the test
4 zones after the distal diffusion front diffuses proximal relative to the test zones.

1 54. A method according to claim 46 wherein the buffer delivered to the buffer
2 addition zone has a volume between about 10 and 250 L.

1 55. A method according to claim 46 wherein the buffer delivered to the buffer
2 addition zone has a volume between about 20 and 200 L.

1 56. A method according to claim 46 wherein the buffer delivered to the buffer
2 addition zone has a volume between about 20 and 100 L.

1 57. A method according to claim 46 wherein the buffer delivered to the buffer
2 addition zone has a volume between about 40 and 60 L.

- 1 58. A method according to claim 46 wherein the buffer delivered to the buffer
2 addition zone comprises the sample delivered to the sample addition zone.
- 1 59. A method according to claim 46 wherein the buffer delivered to the buffer
2 addition zone is the same as the sample delivered to the sample addition zone.
- 1 60. A method according to claim 46 wherein the buffer delivered to the buffer
2 addition zone has substantially the same fluid flow characteristics within the test strip
3 as the sample delivered to the sample addition zone.
- 1 61. A method according to claim 46 wherein the test zones further include a first
2 control zone with a control binding agent immobilized therein, and a second control
3 zone with a same control binding agent immobilized therein as the first control zone,
4 the first and second control zones containing a different amount of the control binding
5 agent immobilized therein.
- 1 62. A method according to claim 46 wherein the test zones further include a first
2 control zone with a control binding agent immobilized therein, and a second control
3 zone with a same control binding agent immobilized therein as the first control zone,
4 the first and second control zones containing about the same amount of the control
5 binding agent immobilized therein.
- 1 63. A method according to claim 46 wherein the test zones further include first
2 and second control zones each with a control binding agent immobilized therein, the
3 first test zone being proximal to the test zone including the first analyte binding agent,
4 the second control zone being distal to the test zone including the first analyte binding
5 agent.